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# Experimental population dynamics of *Rhabdias bufonis* (Nematoda) in toads (*Bufo bufo*): density-dependence in the primary infection

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SUMMARY

Density-dependence in worm establishment, numbers, biomass and larval production were examined in primary infections of 0, 10, 40, 80 and 160 larvae of the lung nematode, *Rhabdias bufonis* in the common toad, *Bufo bufo*. The infection procedure established 4 non-overlapping levels of infection which persisted until 6 weeks post-infection (p.i.), after which there was an overall decline up to 12 weeks p.i. Worm numbers had no direct effect on adult worm survival but temporal changes in worm weight were density-dependent. Adult worm establishment in the lungs declined significantly as the numbers of worms in the lungs increased. At the lowest exposure dose, 86% of the larvae administered reached maturity in the lungs while at the highest, only 37% did so. Also, the numbers of immature larvae outside the lungs increased as adult worm numbers increased. Both features provide evidence for a threshold limit to the numbers of worms maturing in the lungs. Worm numbers also affected larval output per host and *per capita* fecundity. A significant positive relationship between *per capita* fecundity and *per capita* worm weight suggested that density-dependence acted primarily to constrain the growth of individual worms. Finally, the constraints imposed on worm growth and fecundity were apparently relaxed when worm density decreased, providing evidence for density-dependent flexibility in *per capita* fecundity. Density-dependence in worm establishment and *per capita* fecundity are mechanisms which may potentially regulate this host–parasite interaction in the field. Both mechanisms may be functionally related to physical space limitations in the lungs, within which worms must compete for finite nutrients.

Key words: *Rhabdias bufonis*, European toad, *Bufo bufo*, density-dependence, fecundity.

## INTRODUCTION

Experiments on host–parasite systems involving laboratory rodents and their helminth parasites have provided substantial evidence for density-dependent effects on parasite survival, growth and especially fecundity (Anderson, 1982; Keymer, 1982; Keymer & Slater, 1987). Such density-dependent effects play a central role in models of parasite population dynamics (Crofton, 1971; Anderson & May, 1978) and are proposed as key factors in generating stability in natural helminth parasite populations. One of the central conclusions of these experimental systems and models is that helminth abundance is regulated through density-dependent feedback mechanisms which reduce the fitness of individual parasites within hosts as worm density increases. However, the relevance of theoretical studies and of experimental studies involving laboratory-reared hosts and parasites to density-dependent regulation of natural parasite populations is still a matter of controversy (Kennedy, 1975, 1987; Keymer, 1982). The controversy stems largely from a lack of supporting data from natural systems. First, it is difficult to interpret field-collected data in terms of density-dependent regulation (e.g. Anderson & Gordon, 1982; Kennedy, 1984, 1987) and second, it is difficult to bring naturally occurring helminth systems into the laboratory for experimentation.

*Rhabdias bufonis* is a common nematode parasite of European amphibians (Smyth & Smyth, 1980). Adults are hermaphroditic and live in the lungs. Eggs hatch in the intestine, releasing 1st-stage larvae which accumulate in the colon. First-stage homogonic larvae can develop into 3rd-stage larvae in the soil or directly within the faeces. Infective larvae penetrate the skin of definitive hosts, remain in the musculature for a few days, undergo another moult and then migrate through the body cavity to the lungs. The entire life-cycle, from larvae in the faeces, to adults in the lungs, requires a minimum of 12 days. Although the biology of this nematode is well-known from general parasitology literature, it has not been studied experimentally. This study examines the population dynamics of the primary infection of *R. bufonis* in the common toad, *Bufo bufo* and specifically considers the effects of parasite density and time after infection on patterns of worm numbers, survival, mass and fecundity.

## MATERIALS AND METHODS

### *Host and maintenance*

Tadpoles were collected from the Obersee (Kanton Glarus, Switzerland, 990 m altitude) on 26 June 1990. These were collected along a 30 m stretch of shoreline with a hand net. In the laboratory, 20 tadpoles were placed in each of 8, three litre containers and reared with a 3:1 mixture of rabbit chow and Tetramin fish flakes (Alford & Harris, 1988). Water in containers was replaced every 4 days with aged tap water. All metamorphs used in experimental infections emerged between 19 and 24 July. After metamorphosis, 25 toads were placed in each of 7, fifteen litre aquaria and fed *ad libitum* with *Drosophila* for 8 weeks before infection. Individual toads were then acclimated to plastic aquaria (28 × 20 × 10 cm) with perforated lids for a period of 7 days. Each container was provided with fresh water in a 30 mm Petri dish. A moist refuge was also provided by placing, on its side, a small clay flower pot which was immersed in water every 4 days. Containers were checked every 3–4 days for faeces. If present, they were removed in order to prevent re-infection. Containers were thoroughly cleaned once per week.

Toads were maintained on a diet of crickets provided weekly by local animal suppliers. Up to 6 weeks post-infection (p.i.) toads received 0·10 g of 3–5 mm crickets each week (0·05 g twice/week). After 6 weeks p.i. both the mass and size (5–7 mm) of crickets was increased. Every third week, toads received an additional 0·05 g of crickets so that by the end of the experiment each toad received a mean of 0·30 g crickets/week. These food levels (determined by my previous laboratory experience with *Bufo bufo* and *Rana* spp.) always provided food in excess of requirements. After 6 weeks p.i. toads were periodically fed laboratory-reared *Musca* to supplement their diet.

### *Infection procedure*

Infective nematode larvae were obtained from 6 adult toads which had been collected during the breeding season from the Obersee. In the laboratory, adult toads were fed *ad libitum* with crickets and *Musca*. Each toad was naturally infected with *R. bufonis* and released large numbers of 1st-stage larvae in their faeces. Faeces were kept at room temperature on moist filter paper in plastic Petri dishes. Five to 7 days later, large numbers of infective 3rd-stage larvae were active on the surface of the faeces. Once 3rd-stage larvae were present, water was added to Petri dishes containing faeces and the contents were thoroughly mixed. These dishes were then inverted over larger, glass Petri dishes and water was again added. Larvae subsequently escaped from dishes with faeces into the surrounding clean water where they could be easily isolated and counted. These larvae were used for all infection trials and were used within 24 h after leaving the faeces.

In the two low-dose infection treatments, absolute numbers of larvae were counted prior to infection. Dilutions were used in the 2 high dose treatments. In the latter, total numbers of larvae in a 100 ml suspension were estimated in two 0·02 ml aliquot samples. The volume containing the required numbers of larvae was estimated and placed in a 90 mm Petri dish. The numbers of larvae were counted under a dissecting microscope. The numbers of larvae were then adjusted to the required concentration. Suspensions of larvae were then poured over 2 mm filter paper. Filter papers were subsequently placed in 20 mm plastic Petri dishes. Individual toads were confined to these dishes for 24 h. After the infection period, filter papers were washed thoroughly and all remaining larvae were counted.

### *Design and analysis*

Eighty toads were randomly assigned to the individual containers. There were 5 infection treatments (0, 10, 40, 80 and 160 larvae) with 16 replicates. Twenty containers were randomly arranged within each of 4 spatial blocks, each of which contained 4 replicates of each exposure dosage. Each block represented one shelf in a constant temperature room (20 °C; 12:12 light:dark cycle). Two blocks were infected on 22 September and 2 on 24 September. All procedures associated with the infections, host feeding and weighing and the maintenance of containers were conducted according to block to minimize variation among infection treatments.

Within each infection treatment and prior to the beginning of the experiment, toads were randomly assigned to 3 dates for dissection. Four were to be killed at 3 weeks p.i., 4 at 6 weeks p.i. and 8 at the end of the experiment. All toads were killed by immersion in concentrated anaesthetic. Upon dissection, the number and total mass (wet weight in mg) of adult nematodes in the lungs were determined, together with the numbers of immature worms within the body cavity. It was not possible to determine the sex of juvenile toads.

Toads were also assigned to containers according to body mass. Despite the attempted uniformity in growth conditions prior to infection, there was still substantial variation in host weight ($\bar{x}$ = 0·45 g ± 0·18 S.D.). To control for possible bias due to size, the 40 heaviest and 40 lightest toads were separated into 2 containers. When toads were assigned to containers, large ones were selected alternatively with small ones so that each block contained equal numbers of each size class.

Table 1. Summary data of the mean (±S.E.) number of penetrating larvae, number of immature larvae, mean percentage recovery and the mean number of adult *Rhabdias bufonis* recovered from toads after exposure to 4 doses of infective larvae

| | Exposure dosage | | | |
|---|---|---|---|---|
| | 10 | 40 | 80 | 160 |
| No. of penetrating larvae* | | | | |
| $\bar{x}$ | 8·42 (0·48) | 31·54 (1·20) | 63·73 (2·33) | 128·42 (3·23) |
| $\bar{x}$% | 74·40 (4·71) | 78·72 (3·11) | 79·63 (3·03) | 80·22 (2·00) |
| *N* | 16 | 16 | 16 | 16 |
| No. of larvae in body cavity | | | | |
| 3 weeks p.i. | 0 | 0·67 (0·58) | 2·67 (1·16) | 11·25 (7·89) |
| 6 weeks p.i. | 0 | 0 | 0 | 8·00 (8·53) |
| Mean percentage recovery† | | | | |
| $\bar{x}$ | 85·72 (6·51) | 63·40 (5·42) | 51·23 ( 4·04) | 36·92 (2·72) |
| *N* | 7 | 8 | 6 | 6 |
| No. of adult worms | | | | |
| 3 weeks p.i. | | | | |
| $\bar{x}$ | 9·00 | 22·75 | 40·67 | 54·75 |
| S.E. | (±0·58) | (±3·43) | (±4·06) | (±8·04) |
| *N* | 3 | 4 | 3 | 4 |
| 6 weeks p.i. | | | | |
| $\bar{x}$ | 8·75 | 27·50 | 38·67 | 52·5 |
| S.E. | (±1·44) | (±2·36) | (±4·81) | (±9·50) |
| *N* | 4 | 4 | 3 | 2 |
| 12 weeks p.i. | | | | |
| $\bar{x}$ | 6·00 | 8·75 | 19·20 | 5·00 |
| S.E. | (±1·41) | (±3·35) | (±5·46) | (±2·08) |
| *N* | 5 | 4 | 5 | 3 |

* Calculations based on the numbers of larvae remaining in Petri dishes after infection trials.
† Mean percentage of exposure dose which became adult in the lungs.

Larval nematode production was monitored every 3 weeks. The following schedule of host feeding and collection of faeces was used. On Friday, containers were thoroughly cleaned and the toads were fasted on the weekend. The following Monday, toads were fed $\frac{1}{2}$ the total amount of crickets required for the week and on Wednesday the remaining $\frac{1}{2}$. Containers were checked daily for faeces. In most cases, toads deposited faeces in the water dishes. Only faeces which were deposited in water dishes or were completely intact on the floor of the containers were used for analysis. This procedure was followed for 96 h, at which time most toads had deposited one faecal pellet. The contents of each Petri dish were thoroughly mixed and placed in a 100 ml vol. graduated cylinder. Two 0·05 ml aliquots were sampled and the counts averaged to provide an estimate of the total number of larvae produced in 96 h.

Data collected from necropsied hosts were analysed for time and dosage effects by 2-way ANOVA. Analysis of temporal changes in larval production involved sequential counts on specific host individuals. It can therefore not be assumed that data collected over different dates are statistically independent. In such cases I used repeated measures ANOVA (STATVIEW™) for analysis. Proportional data were transformed by the arcsine (square root) prior to analysis and worm mass was log-transformed.

## RESULTS

### *Parasite burden*

Counts of the numbers of larvae remaining after the infection trials showed that the absolute numbers of larvae which penetrated toads (Table 1) was dependent on exposure dose but not on the host's initial body size at infection (exposure dose, $F_{3,57} = 618{\cdot}62$, $P = 0{\cdot}0001$ and size, $F_{1,57} = 2{\cdot}85$, $P = 0{\cdot}097$). The proportion of larvae which penetrated individual toads (Table 1) was not dependent on larval exposure dose but was dependent on the host's initial size at infection (exposure density, $F_{3,57} = 0{\cdot}15$, $P = 0{\cdot}929$ and size, $F_{1,57} = 5{\cdot}86$, $P = 0{\cdot}019$). In general, over 70% of the larvae which were administered,

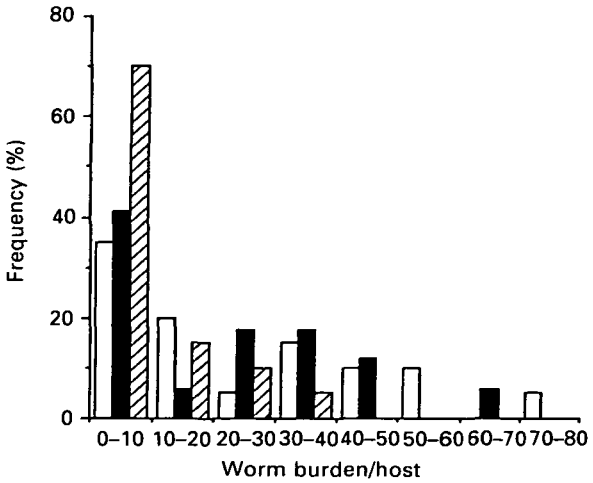


Fig. 1. Frequency distribution of the numbers of adult *Rhabdias bufonis* recovered from toads at necrospy; 3 weeks (□), 6 weeks (■) and 12 weeks (▨) post-infection.

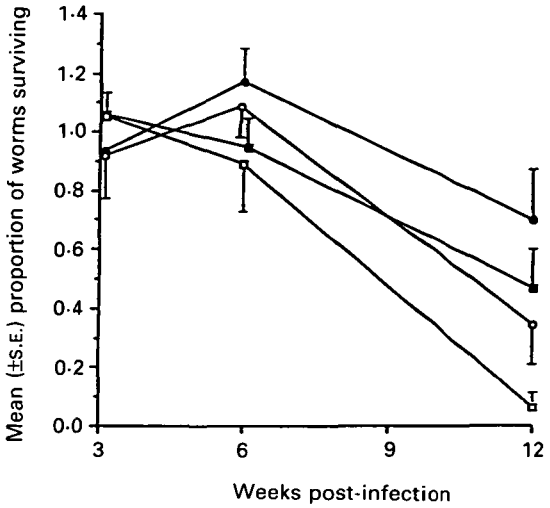


Fig. 2. The survival of *Rhabdias bufonis* in toads exposed to 10 (–●–), 40 (–○–), 80 (–■–) or 160 (–□–) infective larvae. Points represent the mean proportion of worms initially reaching the lungs which were present at 6 and 12 weeks post-infection. Samples sizes are listed in Table 1. Cases in which proportions are greater than 1·0 are those where more adults reached the lungs than expected from the mean percentage of larvae recovered. For example, if mean percentage recovery of 40 larvae is 63·40% (Table 1) then 25 worms would be estimated to initially colonize the lungs. If, at 6 weeks p.i., more than 25 did so, proportional survival is greater than 1·0.

successfully penetrated toads, with a slightly greater proportion penetrating large ($\bar{x} = 82{\cdot}7 \pm 9{\cdot}5$ S.D.) than small ($\bar{x} = 73{\cdot}9 \pm 14{\cdot}6$ S.D.) toads.

All worms recovered from the lungs were sexually mature and contained large numbers of eggs in the uterus. To estimate the maximum numbers of adult worms initially present in the lungs, data on worm burdens at 3 and 6 weeks p.i. were combined. There were no significant differences in worm burden between these two dates ($F_{1,25} = 0{\cdot}0001$, $P = 0{\cdot}977$). Using these pooled data, the percentage recovery (Table 1) of administered larvae which successfully reached maturity in the lungs significantly decreased with exposure dosage but was not dependent on host size (exposure dose, $F_{3,19} = 13{\cdot}60$, $P = 0{\cdot}0001$ and size, $F_{1,19} = 1{\cdot}57$, $P = 0{\cdot}226$). Host size was therefore important only in its influence on the proportion of larvae which penetrated toads and had no influence on the numbers of worms reaching the lungs. This factor was therefore excluded from further analysis. The relationship between percentage recovery and exposure dosage was non-linear ($R^2 = 0{\cdot}677$; $y = 1{\cdot}81x^{-0{\cdot}30}$; D.F. = 26; $P = 0{\cdot}0001$), suggesting there may be a threshold limit to the numbers of larvae which can establish in the lungs. Further evidence for a density threshold is implicated because significantly more immature *R. bufonis* are present at 3 weeks p.i. outside the lungs as exposure dose increased (Table 1, 1-Way ANOVA with exposure dose, $F_{3,12} = 4{\cdot}68$, $P = 0{\cdot}0311$). Moreover, at 6 weeks p.i. immature worms were recovered from the body cavity only at the highest infection dose. Thus, at low exposure doses, over 85% of larvae which penetrated toads, successfully reached the lungs and were gravid by 3 weeks p.i. As dosage increased, an increasingly smaller proportion of the worms which penetrated hosts, reached the lungs. In general, the infection procedure established 4 discrete levels of infection (Table 1) and the relationship between the numbers of larvae which were administered and overall worm numbers was highly significant ($R^2 = 0{\cdot}914$; $y = 1{\cdot}83x^{0{\cdot}70}$, D.F. = 26, $P = 0{\cdot}001$).

### *Parasite survival*

There was a decrease in worm numbers/host between 6 and 12 weeks p.i. at all exposure doses (Table 1). Decrease in worm numbers was accompanied by a decrease in the variation of worm numbers/host; 35% of hosts had between 1 and 10 worms at 3 weeks p.i. while at 12 weeks p.i. the percentage of hosts which were lightly infected approximately doubled (Fig. 1). Age and density-dependent effects on parasite survival were analysed by estimating the proportion of the original numbers of worms reaching the lungs which were present at each of the 3 dates of dissection (Fig. 2). This analysis used mean percentage recovery at each exposure dose (Table 1) as a correction factor. This analysis showed age-dependence in parasite survival but no density-dependence (Table 2).

### *Parasite biomass*

Total parasite biomass/host was also analysed using data collected from hosts necropsied at 3 and 6 weeks p.i. (Fig. 3). There were significant differences in total worm biomass between hosts infected with different doses of larvae (1-Way ANOVA with dose,

Table 2. Summary of ANOVA statistics for effects of time and exposure dose on the survival, biomass and fecundity of *Rhabdias bufonis* in toads

| Response variable | D.F. | MS | *F* | *P* |
|---|---|---|---|---|
| Worm survival | | | | |
| Time | 2 | 2·525 | 31·62 | 0·0001 |
| Exposure dose | 3 | 0·171 | 2·145 | 0·115 |
| Time *X* exposure dose | 6 | 0·136 | 1·697 | 0·155 |
| Error | 31 | 2·476 | | |
| Worm biomass/host | | | | |
| Time | 2 | 1·399 | 33·58 | 0·0001 |
| Exposure dose | 3 | 0·655 | 15·72 | 0·0001 |
| Time *X* exposure dose | 6 | 0·247 | 5·93 | 0·0003 |
| Error | 31 | 0·042 | | |
| *Per capita* worm biomass/host | | | | |
| Time | 2 | 8·917 | 0·98 | 0·386 |
| Exposure dose | 3 | 62·36 | 6·87 | 0·001 |
| Time *X* exposure dose | 6 | 3·936 | 0·43 | 0·850 |
| Error | 31 | 9·072 | | |
| Worm fecundity/host* | | | | |
| Exposure dose | 2 | $3{\cdot}32 \times 10^6$ | 0·80 | 0·486 |
| Error | 7 | $4{\cdot}15 \times 10^6$ | | |
| Time | 3 | $1{\cdot}90 \times 10^6$ | 1·96 | 0·151 |
| Exposure dose *X* time | 6 | $1{\cdot}10 \times 10^6$ | 1·13 | 0·378 |
| Error | 21 | $0{\cdot}097 \times 10^6$ | | |

* Repeated measures ANOVA for differences in numbers of larvae produced/host over 96 h in toads exposed to 10, 40 and 160 larvae.

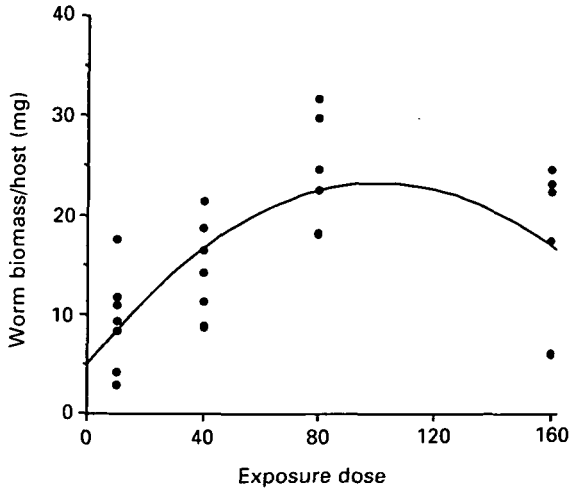


Fig. 3. Total biomass of *Rhabdias bufonis*/host from toads exposed to 4 doses of infective larvae. Points represent biomass determined from toads dissected at 3 and 6 weeks post-infection. The line represents the best fit polynomial regression of total worm biomass and exposure dose ($y = 4{\cdot}27 + 0{\cdot}37x + 1{\cdot}81x^2$, $R^2 = 0{\cdot}43$).

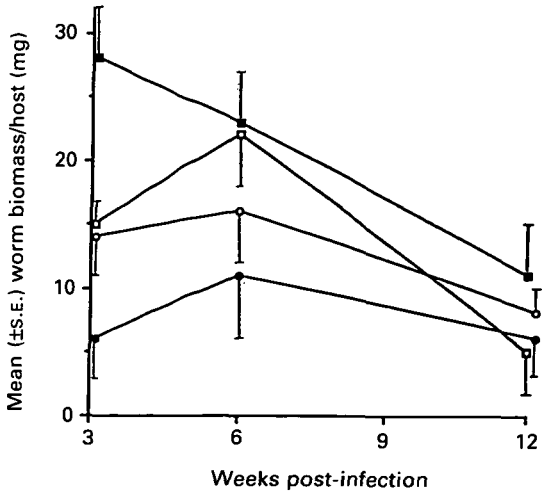


Fig. 4. Mean *Rhabdias bufonis* biomass/host recovered from toads exposed to 10 (-●-), 40 (-○-), 80 (-■-) or 160 (-□-) infective larvae. Points represents mean biomass calculated from toads necropsied at 3 dates of dissection. Samples sizes are listed in Table 1.

$F_{3,25} = 4{\cdot}92$, $P = 0{\cdot}009$). There was also a non-linear relationship between parasite dosage and total worm biomass, with peak biomass occurring at intermediate levels of exposure.

Mean parasite biomass/host (Fig. 4) significantly declined over time and was dependent on exposure dose (Table 2). The interaction between time and exposure dose was also significant. The biomass of worms collected from toads exposed to 10 larvae remained relatively constant throughout the experiment. At higher doses, worm biomass/host converged at 12 weeks p.i. (Fig. 4).

*Per capita* worm mass was strongly dependent on exposure dose but was not age-dependent (Table 2). Individual masses were highest in toads exposed to 10 larvae and lowest in those exposed to 160 larvae (Fig. 5). There was no evidence for convergence in

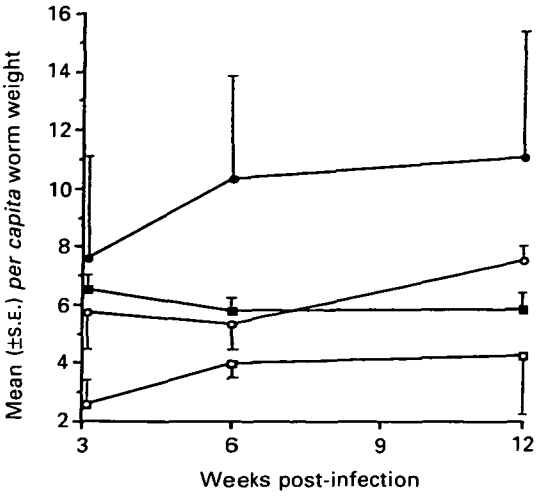


Fig. 5. Temporal changes in mean *per capita* biomass of adult *Rhabdias bufonis*. Points represent mean *per capita* weight of worms recovered from hosts necropsied at 3 dates post-infection. Sample sizes are listed in Table 1. Toads were exposed to 10 (–●–), 40 (–○–), 80 (–■–) or 160 (–□–) infective larvae.

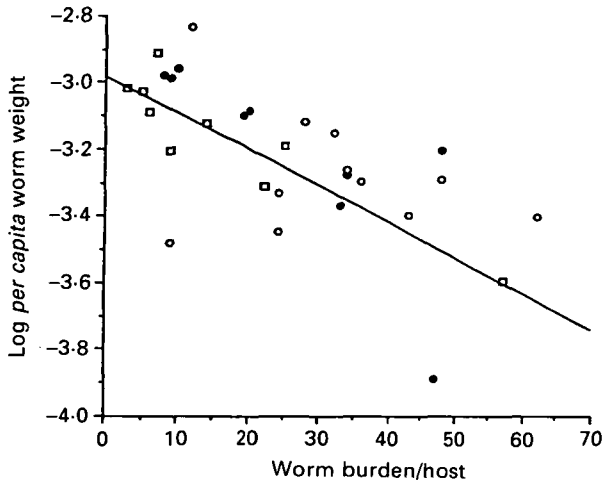


Fig. 6. The relationship between estimates of *per capita* weight of *Rhabdias bufonis* and absolute worm burden at 3 (●), 6 (○) and 12 (□) weeks post-infection. The line represents the best-fit linear regression of $\log_{10}$ worm weight and worm burden ($y = -2{\cdot}99x - 0{\cdot}0009$).

*per capita* worm weight over time indicating that the observed decline in mean worm biomass/host over time (Fig. 5) results from a decrease in worm numbers/host rather than a decrease in the weights of individual worms. Further evidence for density-dependent effects on parasite biomass are indicated by the strong inverse relationship between $\log_{10}$ parasite burden and the estimated weight of individual worms (Fig. 6, D.F. = 28, $t = 4{\cdot}76$, $P = 0{\cdot}0001$).

### *Larval production*

First-stage larvae appeared in the faeces of all infected hosts 12–17 days after infection. There was no relationship between the initial date of larval

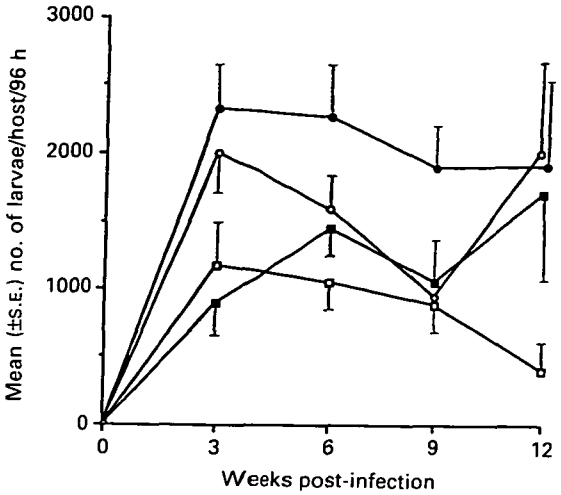


Fig. 7. Temporal changes in the mean numbers of *Rhabdias bufonis* larvae recovered/host in 96 h from toads exposed to 10 (–●–), 40 (–○–), 80 (–■–) or 160 (–□–) infective larvae. Sample sizes of necropsied toads at 3 weeks post-infection from lowest to highest exposure dose – 13, 13, 9, 13 respectively; at 6 weeks – 8, 11, 8, 8; at 9 weeks – 5, 5, 5, 5; at 12 weeks p.i. – 4, 3, 3, 3.

production and exposure dose. There was high variation in the numbers of larvae produced by individual toads, particularly at 12 weeks p.i. when sample sizes were small (Fig. 7). High variation in larval counts/host is a common feature of parasite–host systems and inevitably confounds the analysis of age and density effects (Keymer & Slater, 1987). In this experiment, the most lightly infected toads consistently produced the most larvae (Fig. 7), indicating that density-dependence may be occurring. Analysis of worm fecundity/host was only possible over 3 doses of infection (at $n = 80$ larvae, only 1 toad could be repeatedly monitored for larval production from 3 to 12 weeks p.i.). These data showed no evidence for age-dependence in the numbers of larvae produced by individual toads nor for density-dependence (Table 2). However, repeated measures ANOVA carried out at the highest (160) and lowest (10) doses between 3 and 9 weeks p.i. provided marginal evidence for density-dependent larval production ($F_{1,16} = 5{\cdot}59$, $P = 0{\cdot}045$) but again no evidence for age-dependence ($F_{2,16} = 0{\cdot}712$, $P = 0{\cdot}506$). The difference in larval production between toads exposed to the different doses of larvae were clear at 3 and 6 weeks p.i. but were later obscured by the increased variation (Fig. 7).

*Per capita* fecundity was inversely related to worm burden with extremely high variability among hosts having fewer than 30 worms (Fig. 8). The logarithmic relationship between *per capita* fecundity and worm burden was significantly negative ($R^2 = 0{\cdot}594$, $t = 6{\cdot}28$, D.F. = 28, $P = 0{\cdot}0001$). When *per capita* fecundity was plotted against estimated *per capita* worm mass there was a strong positive association (Fig. 9,

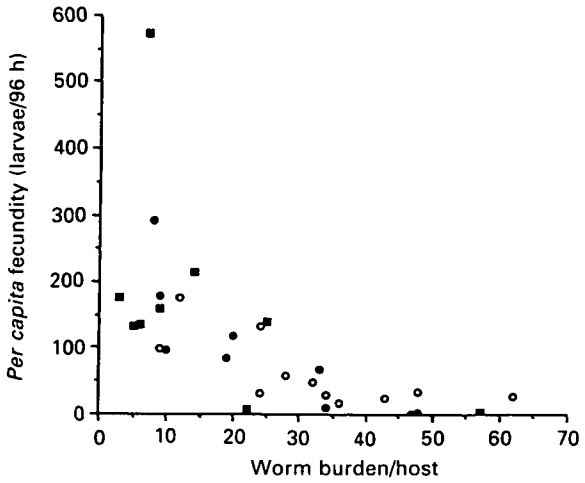


Fig. 8. The relationship between estimates of *per capita* fecundity of *Rhabdias bufonis* and absolute worm burden at 3 (●), 6 (○) and 12 (■) weeks post-infection. Each point represents the estimated *per capita* fecundity of worms in the week prior to necropsy.

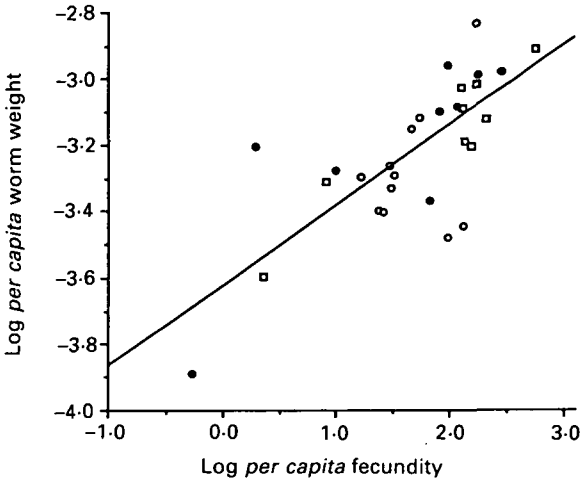


Fig. 9. The logarithmic relationship between estimated *per capita* weight of *Rhabdias bufonis* and *per capita* fecundity at 3 (●), 6 (○) and 12 (■) weeks post infection. Each point represents the estimated *per capita* fecundity of worms in the week prior to necropsy. The line represents the best-fit linear regression of $\log_{10}$ worm weight and $\log_{10}$ worm fecundity ($y = -3{\cdot}62 + 0{\cdot}24$).

$R^2 = 0{\cdot}547$, $t = 5{\cdot}715$, D.F. = 28, $P = 0{\cdot}0001$). This result provides evidence that reductions in helminth fecundity with increasing worm burden may be a consequence of density-dependent constraints on the growth of individual worms.

## DISCUSSION

The data presented here provide strong experimental evidence for density-dependent regulation of a parasite population in its natural host. Specifically, there is evidence for density-dependent reduction in *R. bufonis* establishment in the lungs of toads and for density-dependent reductions in *per capita* worm size and fecundity. These experimental results support the theoretical models of Crofton (1971) and Anderson & May (1978) which have shown that density-dependent reductions in *per capita* worm fitness can play an important role in the regulation of helminths in natural populations.

There are three results which show that establishment of adults in the lungs is density-dependent. First, there was a non-linear, logarithmic relationship between exposure dose and the subsequent number of adult worms in the lungs. This result may indicate a dose-dependent threshold in *R. bufonis* establishment which may be functionally related to physical space limitation in the lungs of toads. The strong convex relationship between exposure dose and total parasite biomass indicates that this threshold acts in particular to constrain total worm biomass rather than worm numbers. Second, the proportion of larvae reaching the lungs decreased non-linearly with exposure dose. Because the proportion of larvae penetrating toads was constant over the 4 infection doses, the decrease in percentage adult recovery must be due to density-dependent mortality of L4 larvae at some stage between penetration and migration to the lungs. Last, as infection dose increased, there were more L4 larvae found outside the lungs. Effectively, all worms from low-dose infections reached maturity in the lungs, but as exposure dose increased, a smaller proportion did so. L4 larvae can survive outside the lungs for up to 6 weeks, but most do not enter the lungs possibly due to limited space within the lung at high densities or to inter-larval competition for openings to the lung.

Worm numbers also affected *per capita* fecundity. The simplest explanation for reduced fecundity is provided by the positive relationship between *per capita* fecundity and *per capita* mass. This relationship, together with the density-dependence in *per capita* worm mass suggests that the decline in worm fecundity at high worm densities is due to decreased growth of worms. These results are in agreement with other laboratory helminth–host systems involving rats or mice as final hosts, in which density-dependent parasite fecundity is correlated with *per capita* worm biomass (Moss, 1971; Chappell & Pike, 1976; Michael & Bundy, 1989).

There is also evidence that reductions in worm growth and fecundity are reversible. The temporal constancy in *per capita* worm weight, despite the decline in worm numbers/host and worm biomass/host suggests that individual worms can compensate for worms which have died by increasing biomass. There is also no concomitant decrease in the numbers of larvae produced/host at 12 weeks p.i. when worm density is lowest. In fact, some of the highest larval counts were obtained from faeces collected during the final week of the experiment. These counts were from toads which were exposed

to moderate levels of infection (40 or 80) but, by necropsy, had lost many of their worms. Thus, it is possible that worm mortality provides conditions in the lungs which enable survivors to maximize growth and fecundity. Similar density-dependent flexibility in *per capita* performance has been suggested for *Angiostrongylus cantonensis* infection in rats (Yong & Dobson, 1982).

There is one caveat to consider before density-dependent reduction in larval production can be unequivocally accepted. Although there is a clear reduction in *per capita* and per host larval production over time, I cannot rule out the possibility that this reduction may be due to a parasite-induced decrease in faecal output. Keymer & Hiorns (1986) showed how a positive association between host faecal deposition and parasite fecundity can obscure the relationship between worm burden and larval output. Such an association could occur if parasites reduce host dietary intake, as has been shown in many parasite–host systems (Crompton, 1984). If reduced diet is associated with reduced faecal output of infected toads, it is possible that reductions in larval output at high parasite density are a result of decreased faecal production by infected toads and not a result of density-dependent larval production. To address this possibility, it would be necessary to monitor larval counts/mass faeces which, in the present system, was not feasible. In any case, it is difficult to conceive how parasite-induced reduction in host faecal output could explain the reductions in *per capita* worm weight observed in this study.

Density-dependent constraints on parasite survival, growth and fecundity are thought to be a result of either intraspecific competition for host resources or interactions with the host's immune response (Anderson & May, 1978; Keymer, 1982). The present data cannot distinguish between these two possibilities. Clearly, there is a finite level of space and nutrients available in the lungs of toads and intra-specific competition for finite resources is a plausible mechanism for density-dependence. Such a mechanism has been proposed by other workers studying anuran helminth–host systems (Jackson & Tinsley, 1988; Tocque & Tinsley, 1991). However, it is well recognized that amphibians possess an immune response comparable to that in mammals (Tinsley, 1989), although it is unknown to what extent it is involved in defence against helminths. More information, particularly involving the response of toads to repeated infection is required before completely discounting the possible impact of immunity on the regulation of *R. bufonis*.

Regardless of initial infection density, almost 90% of toads harboured 1–20 worms by the end of the experiment. Such convergence in parasite numbers (or biomass), when mediated by density-dependent processes, has been repeatedly suggested as a possible mechanism for the regulation of helminth abundance in the field (Anderson & May, 1978; Keymer, 1982). It is noteworthy that populations of adult toads in Europe are approximately 60–100% infected with *R. bufonis* with mean densities of 6–14 worms/host (range 1–183; summarized from 3 studies involving a total of 435 adult toads; Cox, 1971; Kozak, 1973; Frandsen, 1974). Also Plasota (1969) showed that juvenile frogs became infected with *R. bufonis* shortly after metamorphosis and that worm numbers remain relatively constant up to, and throughout, adulthood. These data provide preliminary indications of temporal stability in worm numbers such as that suggested by Keymer (1982) which characterize many helminth–host interactions. Thus, in this system, it is possible that the observed density-dependent constraints which have been shown to occur under experimental conditions may also constrain, and potentially regulate, populations of *R. bufonis* in the field. However, conclusions as to the importance of the observed mechanisms of density-dependence in the laboratory to regulation under natural conditions are speculative. Further experimental studies are required, particularly ones which include hosts (possibly other than *B. bufo*) which are repeatedly exposed to *R. bufonis* and monitored over a longer term.

The conclusions presented in this paper strongly support the existence of density-dependent regulation of parasite population size, probably determined by intra-specific competition for resources. However, it would be difficult to conceive how lung nematodes could compete for host blood resources in a way that suppresses their own size and reproduction, yet not have an impact on host fitness. Indeed, Goater & Ward (1992) have shown that *R. bufonis* has a highly significant effect on host growth and survival and that parasite-induced toad mortality was correlated with parasite burden. Parasite-induced host mortality, which is dose-dependent, is a further mechanism which Anderson & May (1978) and Keymer (1982) have suggested can regulate parasite populations.

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